Influence of Graphene on PCL Scaffolds for Tissue Engineering Applications

Sheila Berna, Guruvel Raja Veluchamy Murugeshan, Isaac Macwan, Prabir Patra
Department of Biomedical Engineering
University of Bridgeport, Bridgeport, CT

Abstract

An important approach in tissue engineering is to develop suitable scaffolds with good properties which is meant for implantation. PCL is a widely used FDA approved polymer in the biomedical field. It is biocompatible, biodegradable and also has good mechanical properties.

We fabricated PCL scaffolds using the electrospinning technique by optimizing various conditions to get bead free nanofibers. Nanofibers produced by electrospinning technique were characterized using Scanning electron microscope (SEM) to confirm the size, surface morphology etc. Graphene is introduced into PCL scaffolds to increase the conductivity of the scaffolds and also to further improve the mechanical properties of the scaffold. These scaffolds provide a good platform for the cells to interact well and proliferate.

Impedance measurements were done for various concentrations of PCL and PCL with graphene. We found that if there is an increase in graphene concentration, there is decrease in impedance measured for the fibers. The future study would be to implant the prepared PCL-Graphene scaffolds into an animal model.

Keywords: PCL, Graphene, Scaffold, Electrospinning

Background

Tissue engineering involves the use of scaffolds, cells, growth factors among which scaffolds are important because it provides structural support and act as template for growth of tissue. Therefore fabrication of scaffolds with appreciable properties and biocompatibility is important. Electrospinning is widely used technique for producing nanofibers. The electrospun nanofibers possesses large surface area to volume ratio, high porosity and good mechanical properties. Moreover, scaffolds produced using electrospinning technique resemble the topographical features of native ECM and supports the growth of cells and regeneration of tissues.

PCL is a non-toxic, biocompatible and biodegradable synthetic polymer. PCL has lower cellular affinity because of its poor hydrophilicity. Performance of PCL can be enhanced by fabricating films of PCL with other biopolymers or nanofillers. Graphene is a single layer of aromatic carbon atoms in a two-dimensional lattice. Graphene has attracted great attention in the preparation of high performance and functional nanofiber scaffolds because of their unique physicochemical, electrical, thermal and biological properties.

In this study, we fabricated the PCL/G composite nanofiber scaffolds by electrospinning and studied the effect of graphene concentration on the conductivity of PCL/G nanofiber scaffolds.

Materials and Methods

Materials necessary for sample preparation of PCL solution w/graphene used for construction of scaffolds were polycaprolactone (PCL), graphene, acetone, a hot plate and a sonicator. Materials necessary for the electrospinning portion of the experiments were a syringe pump, static collector and a voltage source.

In order to make the control PCL samples, the appropriate amount of PCL was weighed out (dependent on the concentration of PCL desired) along with 10mL of acetone. Both the acetone and PCL were then transferred to a vial (w/ cap) with an adequately sized magnetic stir bar and placed on the hot plate. Hot plate was set to the 3¾ setting since it did not have a specific temperature setting and the mixing speed was set to high. By maintaining the temperature and mixing settings for 20-30 minutes, we were able to minimize the amount of solvent being evaporated as well as the amount of product being lost since it was not necessary to run a metal spatula into the contents of the vial for uniform mixing. When making samples containing graphene, solvent and graphene were weighed/measured, transferred into the vial and allowed to mix (w/o heat) for 5-10 minutes. Contents were then sonicated for 15-20 minutes to allow for proper dispersion of graphene into solvent since it is unable to dissolve into solution in the presence of heat. Once sonication was done, polymer was placed into vial and the same mixing and heating procedure was followed for dissolution of polymer in solvent as per instruction used for the control PCL sample.

For electrospinning of solution into scaffolds, 5mL of solution were inserted into a 6mL syringe which was then locked into place on the syringe pump. Due to graphene being a conductive material, the junction locking the syringe needle onto the actual syringe was wrapped with electrical tape so as to avoid sparking during the electrospinning process. Static collector was wrapped with polyethylene film (piece of clear trash bag) which served as the deposition site of the fibers for the formation of the scaffold. Syringe was set to a distance of 15cm from the static collector since that was the optimized distance which allowed for formation of scaffolds without solvent beads present in the scaffold. Voltage ground was attached to the static collector while the input was attached to the tip of the syringe containing the solution. Syringe pump was set to a flow rate of 1.5 mL/hr and voltage source was gradually turned on to 17kV once everything was set in place. Every 5-7 minutes the voltage source was turned off in order to prevent clogging of solution on the tip of the syringe since the solution had the potential to clump and dry up on the tip of the syringe with prolonged exposure to the air. Once scaffold was formed from the 5mL solution, a 3x4 cm section was cut from the scaffold in order to take impedance measurements using electrochemical impedance spectroscopy (EIS).

Results

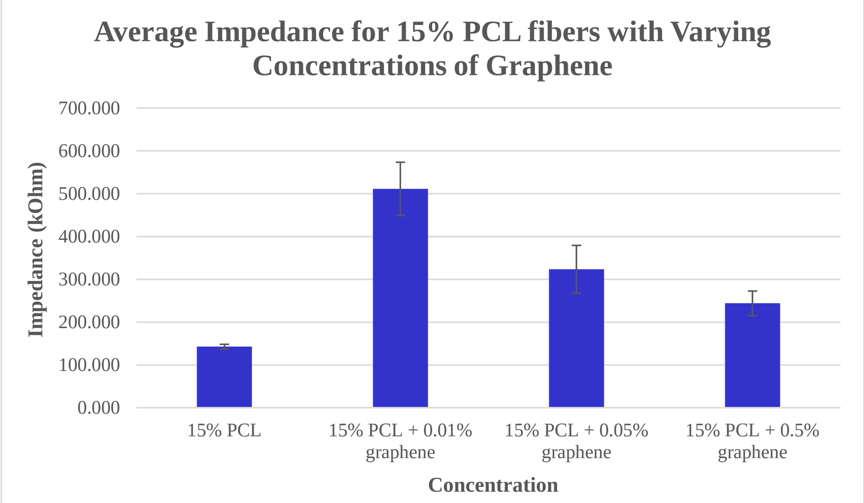

Figure 1. Impedance Values for Scaffolds composed of 15% PCL and varying concentrations of graphene.

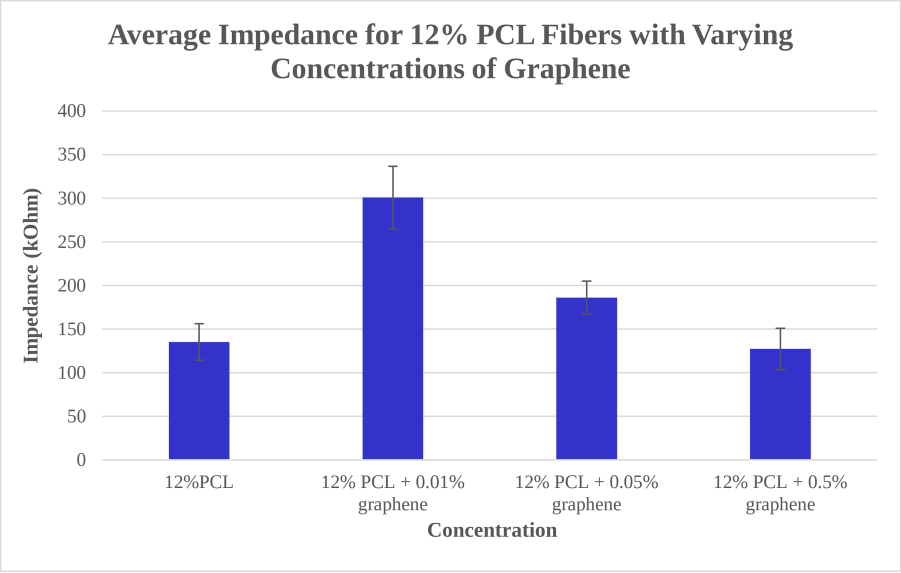

Figure 2. Impedance Values for Scaffolds composed of 12% PCL and varying concentrations of graphene.

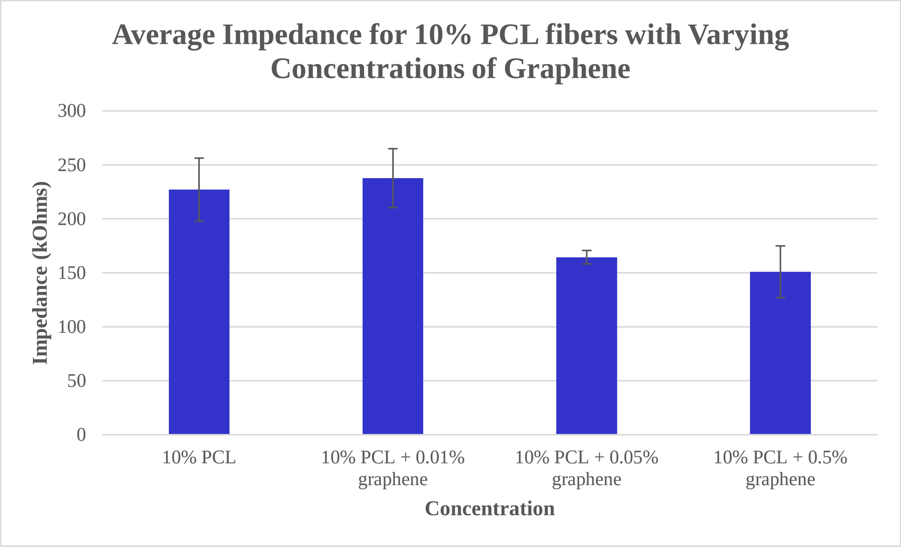

Figure 3. Impedance Values for Scaffolds composed of 10% PCL and varying concentrations of graphene.

Discussion

When analyzing the data as a whole, it is evident that as there is an increase in graphene concentration in the solution for the fibers, there is also a decrease in the impedance measured for the fibers. After tabulating the data, you can clearly see that the 15%, 12% and 10% PCL sample sets show that exact trend although the 10% PCL sample set deviates from showing the same level of consistency. For the 10% PCL set, the average impedance of the 10% PCL (control) is not that far off from the impedance value for the 10% PCL samples with 0.01% graphene; the average impedance for the control sample and the sample with 0.01% graphene only differ at about 4.630%. In the case of the 15% PCL control and 12% PCL control samples versus the 0.01% graphene samples, the percent difference is significantly higher (258% and 123% respectively). In the 15% PCL and 12% PCL set, you clearly see that the PCL control sample tends to fall in the middle (in regards to average impedance) although usually it is lower than that of the sample with 0.01% graphene.

When looking at the data for the samples after grouping together the control, 0.01% graphene, 0.05% graphene and 0.5% graphene samples, you also see that the impedance for the control samples increases. Since PCL is a good insulator and not a good conductor, this is to be expected because the higher the concentration of PCL, the better of an insulator (worse of a conductor) it is and the higher the impedance. This differs from the PCL samples with 0.01% graphene and 0.05% graphene which demonstrate a decrease in impedance with an increase in the concentration of polymer. The comparison for the 0.5% graphene samples showed no consistency at all which furthered the question of whether it would be in our best interest to take replicate measurements for further comparison.

Conclusion

Analysis of impedance data taken for 15%, 12%, and 10% PCL samples with varying concentrations of graphene indicate that as you increase the amount of graphene in each sample, there is a decrease in the impedance for that sample. This is to be expected since impedance is inversely related to conductivity so a decrease in impedance is indicative of an increase in conductivity within the samples. This also indicates that graphene is an effective conductor even when being meshed into a sample containing such a good insulator such a polycaprolactone. Based off of what was observed, it can also be seen that integration of graphene into PCL solution for electrospun scaffolds can provide a good platform for cell growth and proliferation because it will allow cells, such as heart cells, the ability to beat effectively since a conductive surface is being provided for the cells to continue with such and also withstand the stress/strain involved in being incorporated into heart due to PCL's enhanced properties regarding elasticity and response to strain.

References

[1] Aronggaowa, B., Toda, Y., Ito, N., Shikinaka, K., & Shimomura, T. (2013). Transparent Conductive Films Fabricated from Polythiophene Nanofibers Composited with Conventional Polymers. *Polymers*, *5*(4), 1325-1338.
[2] Sirivisoot, S.Pareta, R Harrison, B S. (2014). Protocol and cell responses in three-dimensional conductive collagen gel scaffolds with conductive polymer nanofibres for tissue regeneration. *Interface Focus*, 4(1), 20130050.
[3] Wang, H., Pilon, L. (2012). Intrinsic limitations of impedance measurements in determining electric double layer capacitances. *Electrochimica Acta*, 63, 55-63.
[4] Visser, J., Melchels, F. P., Jeon, J. E., van Bussel, E. M., Kimpton, L. S., Byrne, H. M., ... & Malda, J. (2015). Reinforcement of hydrogels using three-dimensionally printed microfibres. *Nature communications*, *6*.